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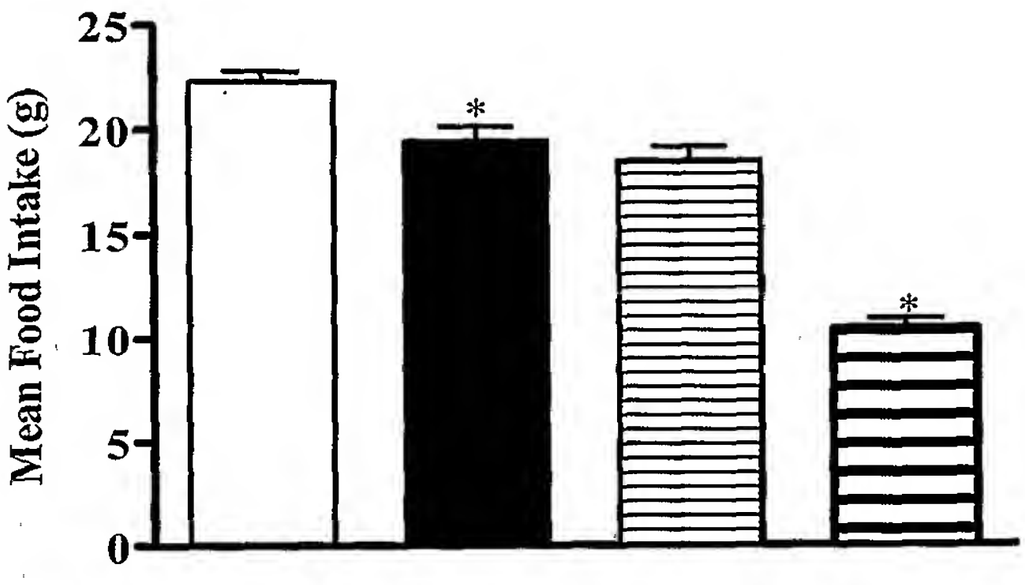
(54) **Title:** METHOD OF TREATMENT OF OBESITY WITH AN MTP INHIBITOR IN CONJUNCTION WITH AN INCREASED-FAT DIET



Mean (± SEM) daily food intake of rats on a high or low fat diet during the 3 day treatment period. Empty bar = low fat vehicle placebo, solid black bar = low fat dirlotapide, small horizontal slashes = high fat vehicle, thick horizontal slashes = high fat dirlotapide. * Significantly different from appropriate diet vehicle.

(57) **Abstract:** A method of treating a subject suffering from obesity or related eating disorders and/or reducing food consumption, the method comprising administering to the subject a therapeutically effective amount of an MTP inhibitor in conjunction with an increased-fat diet.

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METHOD OF TREATMENT OF OBESITY WITH AN MTP INHIBITOR IN CONJUNCTION WITH AN INCREASED-FAT DIET

Field of the Invention

The present invention generally relates to therapy for obesity or related eating disorders and/or reducing food consumption using MTP inhibitors in conjunction with an increased-fat diet.

Background of the Invention

Obesity is a major public health concern because of its increasing prevalence and associated health risks. Moreover, obesity may affect a person's or animal's quality of life through limited mobility and decreased physical endurance as well as through social, academic and job discrimination.

Inhibitors of microsomal triglyceride transfer protein (MTP) and/or Apo B secretion are useful in reducing food intake in mammals (European patent application publication No. 1 099 438 A2), reducing intestinal fat absorption (European patent application publication No. 1 099 439 A2) and for treating obesity and associated diseases. See, for example, PCT patent application publication Nos. WO 03/002533, WO 2005/046644 and WO 2005/080373, and US 6,066, 653.

However, it has been reported in WO 2005/087234 that use of inhibitors of MTP can cause side effects such as hepatotoxicities.

The instant inventors have also found that the MTP inhibitor dirlotapide (disclosed in WO 03/002533) may cause emesis when admininstered according to conventional treatment regimens.

Thus, there is a need to develop more effective methods for treating obesity or related eating disorders and/or reducing food consumption using MTP inhibitors.

Summary of the Invention

The invention provides a method of treating a subject suffering from obesity or related eating disorders and/or reducing food consumption, the method comprising administering to the subject a therapeutically effective amount of an MTP inhibitor in conjunction with an increased-fat diet.

The invention also provides the use of an MTP inhibitor in the manufacture of a medicament for treating a subject suffering from obesity or related eating disorders and/or reducing food consumption, wherein a therapeutically effective amount of an MTP inhibitor is administered in conjunction with an increased-fat diet.

The invention also provides a method of treating a subject suffering from obesity or related eating disorders and/or reducing food consumption, the method comprising administering to the subject a therapeutically effective amount of an MTP inhibitor in conjunction with an increased-fat diet optionally followed by administration of at least one step-wise, escalating dosage of the MTP inhibitor and, optionally, followed by a weight maintenance/management or retraining phase.

The invention also provides the use of an MTP inhibitor in the manufacture of a medicament for treating a subject suffering from obesity or related eating disorders and/or reducing food consumption, wherein a therapeutically effective amount of an MTP inhibitor is administered in conjunction with an increased-fat diet optionally followed by administration of at least one step-wise, escalating dosage of the MTP inhibitor and, optionally, followed by a weight maintenance/management or retraining phase.

The invention also provides a method of treating a subject suffering from obesity or related eating disorders and/or reducing food consumption, the method comprising administering to the subject an MTP inhibitor in conjunction with an increased-fat diet such that the amount of said MTP inhibitor required to be therapeutically effective is reduced compared with conventional treatment regimens.

The invention also provides a method of increasing the rate of weight loss in a subject suffering from obesity or related eating disorders, the method comprising administering to the subject a therapeutically effective amount of an MTP inhibitor in conjunction with an increased-fat diet.

The invention also provides a method of treating a subject suffering from obesity or related eating disorders and/or reducing food consumption, the method comprising administering to the subject an initial amount of an MTP inhibitor effective to ameliorate the obesity or disorder yet low enough to reduce the side effects associated with administration of the MTP inhibitor in conjunction with an increased-fat diet, optionally

followed by administration of at least one step-wise, escalating dosage of the MTP inhibitor and, optionally, followed by a weight maintenance/management or retraining phase.

The invention also provides a method of treating a subject suffering from obesity or related eating disorders and/or reducing food consumption, the method comprising administering to the subject an initial amount of an MTP inhibitor in the range of 0.025 to 0.30 mg/kg/day in conjunction with an increased-fat diet, optionally followed by administration of at least one step-wise, escalating dosage of the MTP inhibitor and, optionally, followed by a weight maintenance/management or retraining phase.

The invention further provides a method for inhibiting MTP in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of an MTP inhibitor in conjunction with an increased-fat diet.

The invention further provides a method of treating a subject suffering from obesity or related eating disorders and/or reducing food consumption, or a method for inhibiting MTP in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of an MTP inhibitor in conjunction with an increased-fat diet, and wherein said administration is in combination with at least one additional pharmaceutical agent, such as another anti-obesity agent.

Also provided is a method of weight control in a subject the method comprising administering to the subject an effective weight-controlling amount of an MTP inhibitor in conjunction with an increased-fat diet. The MTP inhibitor may be used alone or in combination with at least one additional pharmaceutical agent, preferably an anti-obesity agent.

In some embodiments the MTP inhibitor is dirlotapide ((S)-N-{2-[benzyl(methyl)amino]-2-oxo-1-phenylethyl}-1-methyl-5-[4'-trifluoromethyl)[1,1'-biphenyl]-2-carboxamido]-1H-indole-2-carboxamide).

Definitions

Obesity and overweight in humans are generally defined by body mass index (BMI), which is correlated with total body fat and serves as a measure of the risk of certain diseases. BMI is calculated by weight in kilograms divided by height in meters squared (kg/m^2). Overweight is typically defined as a BMI of 25-29.9 kg/m^2, and obesity is typically defined as a BMI of 30 kg/m^2 or higher. Obesity in dogs and cats is usually defined by Body Condition Score (BCS); obesity is >/= 8 and overweight is >/= 6 on a 9-point scale (Purina) or obesity is >/= 5 and overweight is >/= 4 on a 5-point scale (Hill's). The 9-point Purina scale is further discussed in

Laflamme, DP. Body Condition Scoring and Weight Maintenance. *Proc. N. Am. Vet. Conf.* Jan 16-21, 1993. Orlando, FL pp 290-291; and Laflamme DP, Kealy RD. Schmidt, DA. Estimation of Body Fat by Body Composition Score. *J. Vet. Int. Med.* 1994. vol 8, p 154.

Reference to treating obesity included hereinbefore and hereinafter should also be taken to include treatment of overweight subjects.

The phrase "pharmaceutically acceptable" indicates that the substance or composition must be compatible chemically and/or toxicologically, with the other ingredients comprising a formulation, and/or the mammal being treated therewith.

The phrase "therapeutically effective amount" means an amount of a compound that (i) treats or prevents the particular disease, condition, or disorder, (ii) attenuates, ameliorates, or eliminates one or more symptoms of the particular disease, condition, or disorder, or (iii) prevents or delays the onset of one or more symptoms of the particular disease, condition, or disorder described herein (e.g., reduces food intake or the desire to consume food).

The term "subject" or "animal" means humans as well as all other warm-blooded members of the animal kingdom possessed of a homeostatic mechanism, including mammals (e.g., companion animals, zoo animals and food-source animals) and birds. Some examples of companion animals are canines (e.g., dogs), felines (e.g., cats) and horses; some examples of food-source animals are pigs, cows, sheep, poultry and the like. Preferably, the animal is a mammal. Preferably, the mammal is a human, a companion animal or a food-source animal. More preferably, the animal is a human or

is a canine (e.g., a cat or a dog). For example the animal is a canine (e.g. a cat or, preferably, a dog).

The terms "treating", "treat", or "treatment" embrace both preventative, i.e. prophylactic, and palliative treatment.

In the practice of the present invention, the MTP inhibitors are preferably intestinal-acting MTP inhibitors and these are preferably intestinal selective. In this invention, the term "selectivity" refers to a greater effect of a compound in a first assay, compared to the effect of the same compound in a second assay. In the above embodiment of the invention, the first assay is for the ability of the compound to inhibit intestinal fat absorption and the second assay is for the ability of the compound to lower serum triglycerides. In a preferred embodiment, the ability of the compound to inhibit intestinal fat absorption is measured by the dose that produces 25% fat absorption inhibition (ED_{25}) of the compound in an intestinal fat absorption assay, such that a greater effect of the compound results in the observation of a lower absolute (numerical) value for the ED_{25}. In another preferred embodiment, the ability of the compound to lower serum triglycerides is measured by the ED_{25} of the compound in a serum triglyceride assay. Again, a greater effect of a compound in the serum triglyceride lowering assay results in the observation of a lower absolute (numerical) value for the ED_{25}. It is to be understood that any assay capable of measuring the effectiveness of a compound in inhibiting intestinal fat absorption, or capable of measuring the effectiveness of a compound in lowering serum triglycerides, is encompassed by the present invention. Examples of suitable assays are given in PCT Publication No. WO 03/002533.

Intestinal selectivity may be achieved by controlling the solubility of the inhibitor in the intestinal tract and/or release of the inhibitor from the dosage form or by increasing lipid (fat) in the gut, i.e. administer with food and increase the dietary fat in the food.

Brief Description of the Drawings

Figure 1 and Figure 2 relate to a study to show efficacy and safety of dirlotapide at a starting dose of 0.1 mg/kg in the treatment of excessive body weight in overweight Labrador dogs fed diets with varying fat contents for up to 52 weeks –

Figure 1 provides a summary of the dose volumes administered throughout the study period;

Figure 2 provides a summary of the mean cumulative body weight change (%) during the weight loss phase;

Figure 3 provides a summary of the mean daily food intake in rats treated with (4'-trifluoromethyl-biphenyl-2-carboxylic acid [2-(2H-[1,2,4]triazol-3-ylmethyl)-1,2,3,4-tetrahydro-isoquinolin-6-yl] amide) at 10, 30 or 100mg/kg or vehicle with diets of 45% (A) or 10% (B) of total calories from fat;

Figure 4 provides a summary of the mean daily food intake in rats treated with dirlotapide at 10mg/kg or vehicle with "high" or "low" fat diets.

Detailed Description of the Invention

Applicants have discovered that, although MTP inhibitors are effective anti-obesity agents when used with any diet, the efficacy of MTP inhibitors as anti-obesity agents is significantly enhanced when the MTP inhibitor is administered in conjunction with an increased-fat diet. Thus, it has been found that weight loss can be achieved with lower doses of MTP inhibitor when the subject is fed with a higher-fat diet as opposed a lower-fat diet. Subjects also lost weight at a higher rate when the MTP inhibitor was administered in conjunction with an increased-fat diet. In addition, significant reductions in food intake can be achieved when the MTP inhibitor is administered in conjunction with an increased-fat diet.

The term "increased-fat diet" or "high-fat diet" or similar when used in connection with the present invention means a diet having a fat content of approximately 10% or greater than 10%, i.e. at least approximately 10% (based on air dry matter as fed).

"Air dry matter as fed" relates to the air dry form of the feed as fed which typically includes around 10% moisture in dry dog food. Therefore, for example, 10% fat based on air dry matter as fed would be equivalent to approximately 11% fat on a dry matter basis and 15% fat based on air dry matter as fed would be equivalent to approximately 16.7% fat on a dry matter basis.

Thus, the term "increased-fat diet" includes those conventional pet (e.g. dog) foods marketed as typical fat or maintenance diets (having fat contents, for example, in the range of 10 to 17%) and those conventional pet (e.g. dog) foods marketed as high fat or active or performance diets (having fat contents, for example, greater than 18%) both based on air dry matter as fed.

Alternatively, diets can be defined by the percentage of metabolizable energy (Kcal) they provide from fat (Kcal). Thus for example, the term "increased-fat diet" or similar when used in connection with the present invention means a diet providing approximately 24% or greater than 24% of total calories from fat, such as approximately 34% or greater than 34% of total calories from fat, such as approximately 45% or greater than 45% of total calories from fat. Thus, the term "increased-fat diet" includes those conventional pet (e.g. dog) foods marketed as typical fat or maintenance diets (providing approximately, for example, 24 to 33% total calories from fat) and those conventional pet (e.g. dog) foods marketed as high fat or active or performance diets (providing approximately, for example, 34 to 50% total calories from fat).

As a particular example, the term "increased-fat diet" or similar when used in connection with the present invention means a diet having a fat content of approximately 15% or greater than 15%, i.e. at least approximately 15% (based on air dry matter as fed). As a further particular example, the term "increased-fat diet" or similar when used in connection with the present invention means a diet providing approximately 45% or greater than 45%, i.e. at least approximately 45% of total calories from fat.

MTP inhibitors for use in the present invention are known in the art. Suitable MTP inhibitors include compounds disclosed in U.S. Patent Nos. 4,453,913; 4,473,425; 4,491,589; 4,540,458; 4,962,115; 5,057,525; 5,137,896; 5,286,647; 5,521,186; 5,595,872; 5,646,162; 5,684,014; 5,693,650; 5,712,279; 5,714,494; 5,721,279; 5,739,135; 5,747,505; 5,750,783; 5,760,246; 5,789,197; 5,811,429; 5,827,875;

5,837,733; 5,849,751; 5,883,099; 5,883,109; 5,885,983; 5,892,114; 5,919,795; 5,922,718; 5,925,646; 5,929,075; 5,929,091; 5,935,984; 5,952,498; 5,962,440; 5,965,577; 5,968,950; 5,998,623; 6,025,378; 6,034,098; 6,034,115; 6,051,229; 6,051,387; 6,051,693; 6,057,339; 6,066,650; 6,066,653; 6,114,341; 6,121,283; 6,191,157; 6,194,424; 6,197,798; 6,197,972; 6,200,971; 6,235,730; 6,235,770; 6,245,775; 6,255,330; 6,265,431; 6,281,228; 6,288,234; 6,329,360; 6,342,245; 6,369,075; 6,417,362; 6,451,802; 6,479,503; 6,492,365; 6,583,144; 6,617,325; 6,713,489; 6,720,351; 6,774,236; 6,777,414; and 6,878,724:

US Patent Publication Nos. 2002/028940; 2002/032238; 2002/055635; 2002/132806; 2002/147209; 2003/149073; 2003/073836; 2003/105093; 2003/114442; 2003/0162788; 2003/166590; 2003/166637; 2003/181714; 2004/009988; 2004/014971; 2004/024215; 2004/034028; 2004/044008; 2004/058903; 2004/102490; 2004/157866; and 2005/234099:

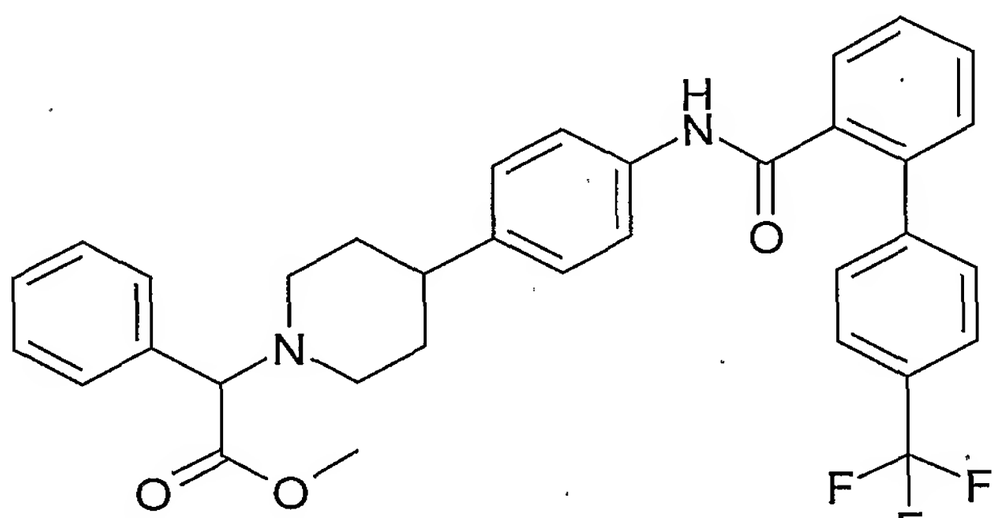
PCT Patent Publication Nos. WO 96/262205; WO 98/016526; WO 98/031366; WO99/55313; WO 00/005201; WO 01/000183; WO 01/000184; WO 01/000189; WO 01/005767; WO 01/012601; WO 01/014355; WO 01/021604; WO 01/053260; WO 01/074817; WO 01/077077; WO 02/014276; WO 02/014277; WO 02/081460; WO 02/083658; WO 04/017969; and WO05/080373: and

Japanese Patent Publication Nos. JP2002-212179(14212179); and JP2002-220345(14220345).

For a review of apo-B/MTP inhibitors, see, Williams, S.J. and J.D. Best, Expert Opin Ther Patents, **13**(4), 479-488 (2003). For methods that may be used to identify active MTP inhibitors, see, Chang, G., et al., "Microsomal triglyceride transfer protein (MTP) inhibitors: Discovery of clinically active inhibitors using high-throughput screening and parallel synthesis paradigms," Current Opinion in Drug Discovery & Development, **5**(4), 562-570 (2002). All of the above patents, patent applications and references are incorporated herein by reference.

Preferred intestinal-acting MTP inhibitors for use in the instant invention include dirlotapide ((S)-N-{2-[benzyl(methyl)amino]-2-oxo-1-phenylethyl}-1-methyl-5-[4'-trifluoromethyl)[1,1'-biphenyl]-2-carboxamido]-1H-indole-2-carboxamide)

and 1-methyl-5-[(4'-trifluoromethyl-biphenyl-2-carbonyl)-amino]-1H-indole-2-carboxylic acid (carbamoyl-phenyl-methyl)-amide which can both be prepared using methods described in U.S. Patent No. 6,720,351; (S)-2-[(4'-trifluoromethyl-biphenyl-2-carbonyl)-amino]-quinoline-6-carboxylic acid (pentylcarbamoyl-phenyl-methyl)-amide, (S)-2-[(4'-tert-butyl-biphenyl-2-carbonyl)-amino]-quinoline-6-carboxylic acid {[(4-fluoro-benzyl)-methyl-carbamoyl]-phenyl-methyl}-amide, (S)-2-[(4'-tert-butyl-biphenyl-2-carbonyl)-amino]-quinoline-6-carboxylic acid [(4-fluoro-benzylcarbamoyl)-phenyl-methyl]-amide, and (S)-2-[(4'-isopropoxy-biphenyl-2-carbonyl)-amino]-quinoline-6-carboxylic acid {[(4-fluoro-benzyl)-methyl-carbamoyl]-phenyl-methyl}-amide which can all be prepared as described in U.S. Publication No. 2005/0234099; (-)-4-[4-[4-[4-[[(2*S*,4*R*)-2-(4-chlorophenyl)-2-[[(4-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]methyl-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]phenyl]-2-(1*R*)-1-methylpropyl]-2,4-dihydro--3*H*-1,2,4-triazol-3-one (also known as Mitratapide or R103757) which can be prepared as described in U.S. Patent Nos. 5,521,186 and 5,929,075; implitapide (BAY 13-9952) which can be prepared as described in U.S. Patent No. 6,265,431; R256918 which has the structure



and can be prepared as described in U.S. Patent No. 6,878,724; and (4'-trifluoromethyl-biphenyl-2-carboxylic acid [2-(2H-[1,2,4]triazol-3-ylmethyl)-1,2,3,4-tetrahydro-isoquinolin-6-yl] amide). Most preferred is dirlotapide, mitratapide, (S)-2-[(4'-trifluoromethyl-biphenyl-2-carbonyl)-amino]-quinoline-6-carboxylic acid (pentylcarbamoyl-phenyl-methyl)-amide, (S)-2-[(4'-tert-butyl-biphenyl-2-carbonyl)-amino]-quinoline-6-carboxylic acid {[(4-fluoro-benzyl)-methyl-carbamoyl]-phenyl-methyl}-amide, (S)-2-[(4'-tert-butyl-

biphenyl-2-carbonyl)-amino]-quinoline-6-carboxylic acid [(4-fluoro-benzylcarbamoyl)-phenyl-methyl]-amide, (S)-2-[(4'-isopropoxy-biphenyl-2-carbonyl)-amino]-quinoline-6-carboxylic acid {[(4-fluoro-benzyl)-methyl-carbamoyl]-phenyl-methyl}-amide, R256918, or (4'-trifluoromethyl-biphenyl-2-carboxylic acid [2-(2H-[1,2,4]triazol-3-ylmethyl)-1,2,3,4-tetrahydro-isoquinolin-6-yl] amide).

In a preferred embodiment the MTP inhibitor for use in the methods of the present invention is (4'-trifluoromethyl-biphenyl-2-carboxylic acid [2-(2H-[1,2,4]triazol-3-ylmethyl)-1,2,3,4-tetrahydro-isoquinolin-6-yl] amide, or more preferably, the compound dirlotapide.

For human use, the conventional daily dose of the intestinal-acting MTPi is generally between about 0.05 mg to about 50 mg, preferably between about 0.5 mg to about 30 mg, more preferably between about 0.5 mg to about 20 mg, most preferably between about 1.0 mg to about 15 mg. For non-human use, those skilled in the art know how to adjust the dosage for the particular weight of the animal. In some circumstances, the MTPi may be administered in combination with an agent to reduce fatty liver (e.g., fibrate or PPAR-alpha agonist). See, e.g., JP Publication No. 2002-220345 (Application No. 2001-015602) entitled "Remedial Agent for Fatty Liver"; and Kersten, S., "Peroxisome Proliferator Activated Receptors and Obesity," Eur J Pharm, **440**, 223-234 (2002).

In some embodiments, the MTP inhibitor is administered at escalating dosages. In some embodiments, the escalating dosages comprise at least an initial first dose level and a second dose level. In some embodiments, the escalating dosages comprise at least a first dose level, a second dose level and a third dose level. In some embodiments, the escalating dosages further comprise a fourth dose level. In some embodiments, the escalating dosages comprise at least a first dose level, a second dose level, a third dose level, a fourth dose level and a fifth dose level. In some embodiments, six and further dose levels are contemplated.

The original dose level may be increased by 10 %, 20%, 25%, 50%, 100% or 300% to produce the next dose level. When the original dose level is increased by 100%, the next dose level is double the original dose level. When the original dose level is increased by 300%, the next dose level is four times the original dose level. In

some embodiments the original dose level is increased by 25%, 50% or 100%. Preferably, the original dose level is increased by 50% or 100%, for example 100%.

Preferably, the first dose level is in the range of 0.025 to 0.30 mg/kg/day, for example in the range of 0.025 to 0.10 mg/kg/day, such as about 0.05 or 0.10 mg/kg/day, preferably about 0.05 mg/kg/day.

Preferably, the second dose level is 100% greater than the first, for example is in the range of 0.05 to 0.6 mg/kg/day, or for example is in the range of 0.05 to 0.2 mg/kg/day, such as about 0.10 or 0.2 mg/kg/day, preferably about 0.10 mg/kg/day.

Preferably, the third dose level is 100% greater than the second dose level, for example is in the range of 0.10 to 1.2 mg/kg/day, or for example is in the range of 0.10 to 0.4 mg/kg/day, for example about 0.2 or 0.4 mg/kg/day, preferably about 0.2 mg/kg/day.

Preferably, the fourth dose level is 50% greater than the third dose level, for example is in the range of 0.15 to 0.9 mg/kg/day, or for example is in the range of 0.15 to 0.6 mg/kg/day, for example about 0.3 or 0.6 mg/kg/day, preferably about 0.3 mg/kg/day.

Preferably, the fourth dose level is increased by 50% thereafter to produce fifth, six and subsequent dose levels.

When the MTP inhibitor is dirlotapide, preferably the first dose level is in the range of 0.025 to 0.10 mg/kg/day, for example about 0.05 or 0.10 mg/kg/day, preferably about 0.05 mg/kg/day. Preferably, the second dose level is 100% greater than the first, for example is in the range of 0.05 to 0.2 mg/kg/day, for example about 0.10 or 0.2 mg/kg/day, preferably about 0.10 mg/kg/day. Preferably, the third dose level is 100% greater than the second dose level, for example is in the range of 0.10 to 0.4 mg/kg/day, for example about 0.2 or 0.4 mg/kg/day, preferably about 0.2 mg/kg/day. Preferably, the fourth dose level is 50% greater than the third dose level, for example is in the range of 0.15 to 0.6 mg/kg/day, for example about 0.3 or 0.6 mg/kg/day, preferably about 0.3 mg/kg/day. Preferably, the fourth dose level is increased by 50% thereafter to produce fifth, six and subsequent dose levels.In some embodiments, each dose level is administered to the subject for from about 1 to 4 weeks, for example, the dose levels may be increased after 1 week, 2 weeks, or monthly. For example, the first dose level,

e.g. 0.05 mg/kg/day, may be administered for about 14 days, and then the second dose level, e.g. 0.01 mg/kg/day, may be administered for about 14 days, and then the third dose level, e.g. 0.2 mg/kg/day, may be administered for about a month, with subsequent dose increases being made at, for example, monthly intervals.

For example, when the MTP inhibitor is dirlotapide and the subject is a dog, the initial dose may be 0.05 mg/kg/day. After two weeks of therapy, the initial dose may be doubled to 0.10 mg/kg/day for two weeks. Following these initial 4 weeks of therapy, dogs may be weighed monthly and dose adjustments may be made monthly according to the following guidelines. At the end of each month of therapy, the percentage of body weight loss is determined. If the body weight loss since previous monthly weighing has been greater than or equal to 3% body weight per month (equivalent to 0.1% body weight per day); the dose may be kept the same. If the body weight loss since previous monthly weighing has been less than 3% body weight per month; the dose may be increased without adjusting for the dog's current body weight. The first time a conditional increase is required, the dose may be increased by 100% (doubled). In subsequent required conditional increases, the dose may be increased by 50% up to a maximum dose of the product of 1 mg/kg current body weight. These adjustments may be continued until the weight targeted at the start of therapy is achieved.

In cases where body weight loss since previous monthly weighing has been greater than or equal to 12% per month, the dose may be reduced by 25%.

A mean weight loss of about 18 to 20% after six months of weight loss therapy can be anticipated.

The initial "weight loss" phase may last a number of months, for example about 4 months (i.e. about 16 weeks) to 6 months, or for example, about 112 to 196 days, or may last until the target weight loss is achieved, or may last until a particular Body Condition Score (BCS) is reached, for example a BCS of five.

The weight maintenance/management or retraining phase may last for a period of months, for example about 3 months (i.e. about 12 weeks) or, for example, 84 days. During the retraining phase, the dose may be decreased, for example by 50%, or increased, for example by 100%, if the patient was losing or gaining too much weight

(for example, more than 5%) from the start of the weight maintenance/management retraining phase, respectively.

As mentioned above, when the desired weight is reached, the weight maintenance/management or retraining phase can be commenced. During the weight maintenance/management or retraining phase the optimal level of food intake and physical activity needed should be established. Administration of the MTP inhibitor should be continued during the weight maintenance/management or retraining phase until the food intake and physical activity needed to stabilize body weight at the desired weight is established.

For example, when the MTP inhibitor is dirlotapide and the subject is a dog, the dose adjustment during the weight maintenance/management or retraining phase may be as follows:

First dose adjustment

If the dog lost greater than or equal to 1% body weight per week in the last month of the weight loss phase, the dose should be decreased by 50%.

If the dog lost between 0 and 1% the dose should remain the same.

If the dog gained weight, the dose should be increased by 50%.

Subsequent dose adjustments

In subsequent months the dose should be increased or decreased by 25% to maintain a constant weight.

If the dog is within -5% to +5% of the body weight at the end of the weight loss phase, the dose should remain unchanged.

If the dog lost greater than 5% body weight, then the dose should be decreased by 25%.

If the dog gained greater than 5% body weight, then the dose should be increased by 25%. Based on the dog's current body weight a daily dose of 1 mg/kg should not be exceeded.

When the drug is discontinued, the daily amount of food offered and physical activity should be continued as established during the weight maintenance/management or retraining phase.

As used herein the term "reduce the side effects associated with administration of the MTP inhibitor" or similar refers to an amelioration or elimination of one or more undesired side effects occurring as a result of administering MTP inhibitors according to traditional treatment regimens, for example at higher initial doses without dose escalation and without being administered in conjunction with an increased-fat diet. Such side effects include emesis (vomiting), diarrhoea, lethargy, inappetence and anorexia, for example emesis (vomiting), diarrhea and lethargy and particularly include emesis.

In some embodiments the methods further comprise the administration of at least one additional pharmaceutical agent. Suitable additional pharmaceutical agents include other anti-obesity agents such as cannabinoid-1 (CB-1) antagonists (such as rimonabant), 11β-hydroxy steroid dehydrogenase-1 (11β-HSD type 1) inhibitors, peptide YY (PYY) and PYY agonists (such as PYY_{3-36} or analogs or derivatives thereof), MCR-4 agonists, cholecystokinin-A (CCK-A) agonists, monoamine reuptake inhibitors (such as sibutramine), sympathomimetic agents, β_3 adrenergic receptor agonists, dopamine receptor agonists (such as bromocriptine), melanocyte-stimulating hormone receptor analogs, 5HT2c receptor agonists, melanin concentrating hormone antagonists, leptin (the OB protein), leptin analogs, leptin receptor agonists, galanin antagonists, lipase inhibitors (such as tetrahydrolipstatin, i.e. orlistat), anorectic agents (such as a bombesin agonist), neuropeptide-Y receptor antagonists (e.g., NPY Y5 receptor antagonists), thyromimetic agents, dehydroepiandrosterone or an analog thereof, glucocorticoid receptor agonists or antagonists, orexin receptor antagonists, glucagon-like peptide-1 receptor agonists, ciliary neurotrophic factors (such as Axokine™ available from Regeneron Pharmaceuticals, Inc., Tarrytown, NY and Procter & Gamble Company, Cincinnati, OH), human agouti-related protein (AGRP) inhibitors, ghrelin receptor antagonists, histamine 3 receptor antagonists or inverse agonists, neuromedin U receptor agonists and the like.

Other suitable pharmaceutical agents include lipid modifying compounds which include HMG CoA reductase inhibitors, cholesterol absorption inhibitors, ezetimide, squalene synthetase inhibitors, fibrates, bile acid sequestrants, statins, probucol and

derivatives, niacin, niacin derivatives, PPAR alpha agonists, PPAR gamma agonists, thiazolidinediones, and cholesterol ester transfer protein (CETP) inhibitors.

Other suitable additional pharmaceutical agents include LDL-cholesterol lowering agents, triglyceride lowering agents, an HMG-CoA reductase inhibitor, an HMG-CoA synthase inhibitor, an inhibitor of HMG-CoA reductase gene expression, a squalene synthetase inhibitor, a squaline epoxidase inhibitor, a squaline cyclase inhibitor, a combined squaline epoxidase/cyclase inhibitor, a cholesterol synthesis inhibitor, a cholesterol absorption inhibitor such as Zetia™ (ezetimibe), a CETP inhibitor, a PPAR modulator or other cholesterol lowering agent such as a fibrate, an ion-exchange resin, an antioxidant, an ACAT inhibitor or a bile acid sequestrant. Other pharmaceutical agents useful in the practice of the combination aspect of the invention include bile acid reuptake inhibitors, ileal bile acid transporter inhibitors, ACC inhibitors, antihypertensive agents (such as Norvasc®), diuretics, garlic extract preparations, bile acid sequestrants, antibiotics, antidiabetics, and anti-inflammatory agents such as aspirin or, preferably, an anti-inflammatory agent that inhibits cyclooxygenase-2 (Cox-2) to a greater extent than it inhibits cyclooxygenase-1 (Cox-1) such as celecoxib (U.S. patent No. 5,466,823), valdecoxib (U.S. patent No. 5,633,272, parecoxib (U.S. patent No. 5,932,598), deracoxib (CAS RN 169590-41-4), rofecoxib ((CAS RN 162011-90-7), etoricoxib (CAS RN 202409-33-4), lumiracoxib (CAS RN 220991-20-8) or carprofen (CAS RN 53716-49-7).

Other suitable additional pharmaceutical agents include naturally occurring substances that act to lower plasma cholesterol levels. These naturally occurring materials are commonly called nutraceuticals and include, for example, garlic extract, *Hoodia* plant extracts and niacin.

The dosage of the additional pharmaceutical agent is generally dependent upon a number of factors including the health of the subject being treated, the extent of treatment desired, the nature and kind of concurrent therapy, if any, and the frequency of treatment and the nature of the effect desired. In general, the dosage range of the additional pharmaceutical agent is in the range of from about 0.001 mg to about 100 mg per kilogram body weight of the individual per day, preferably from about 0.1 mg to about 10 mg per kilogram body weight of the individual per day. However, some

variability in the general dosage range may also be required depending upon the age and weight of the subject being treated, the intended route of administration, the particular anti-obesity agent being administered and the like. The determination of dosage ranges and optimal dosages for a particular patient is also well within the ability of one of ordinary skill in the art having the benefit of the instant disclosure.

In some embodiments, the additional pharmaceutically active agents are administered according to traditional treatment regimens. In some embodiments, the additional pharmaceutically active agents are administered at escalating dosages.

According to the methods of treatment of the invention, the MTP inhibitor and any additional pharmaceutical agent (referred to herein as a "combination") is administered to a subject in need of such treatment, preferably in the form of a pharmaceutical composition. In the combination aspect of the invention, the MTP inhibitor and the other pharmaceutical agent (e.g., another anti-obesity agent,) may be administered either separately or in a pharmaceutical composition comprising both. It is generally preferred that such administration be oral. When a combination of MTP inhibitor and any other pharmaceutical agent are administered together, such administration may be sequential in time or simultaneous. Simultaneous administration of drug combinations is generally preferred. For sequential administration, the agents may be administered in any order. It is generally preferred that such administration be oral. It is especially preferred that such administration be oral and simultaneous. When the MTP inhibitor and the additional pharmaceutical agent are administered sequentially, the administration of each may be by the same or by different methods.

According to the methods of the invention, the MTP inhibitor or a combination is preferably administered in the form of a pharmaceutical composition. Administration of the agents can be separately or together in any conventional oral, rectal, transdermal, parenteral (e.g., intravenous, intramuscular or subcutaneous), intracisternal, intravaginal, intraperitoneal, topical (e.g., powder, ointment, cream, spray or lotion), buccal or nasal dosage form (e.g., spray, drops or inhalant).

The MTP inhibitors or combinations can be administered alone but will generally be administered in an admixture with one or more suitable pharmaceutical excipients, adjuvants, diluents or carriers known in the art and selected with regard to the intended

route of administration and standard pharmaceutical practice. The MTP inhibitors or combination may be formulated to provide immediate-, delayed-, modified-, sustained-, pulsed- or controlled-release dosage forms depending on the desired route of administration and the specificity of release profile, commensurate with therapeutic needs.

The pharmaceutical composition comprises an MTP inhibitor or a combination in an amount generally in the range of from about 1% to about 75%, 80%, 85%, 90% or even 95% (by weight) of the composition, usually in the range of about 1%, 2% or 3% to about 50%, 60% or 70%, more frequently in the range of about 1%, 2% or 3% to less than 50% such as about 25%, 30% or 35%.

Methods of preparing various pharmaceutical compositions with a specific amount of active compound are known to those skilled in this art. For examples, see Remington: The Practice of Pharmacy, Lippincott Williams and Wilkins, Baltimore MD, 20th ed. 2000.

Examples of suitable formulations are further described in, for example, WO 03/002533, WO 2005/046644 and WO2005/080373.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs. In addition to the MTP inhibitor or the combination, the liquid dosage form may contain inert diluents commonly used in the art, such as water or other solvents, solubilizing agents and emulsifiers, as for example, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (e.g., cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, sesame seed oil and the like), Miglyol® (available from CONDEA Vista Co., Cranford, NJ.) , glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, or mixtures of these substances, and the like.

Besides such inert diluents, the composition may also include excipients, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Oral liquid forms of the MTP inhibitors or combinations include solutions, wherein the active compound is fully dissolved. Examples of solvents include all pharmaceutically precedented solvents suitable for oral administration, particularly those

in which the compounds of the invention show good solubility, e.g., polyethylene glycol, polypropylene glycol, edible oils and glyceryl- and glyceride- based systems. Glyceryl- and glyceride- based systems may include, for example, the following branded products (and corresponding generic products): Captex™ 355 EP (glyceryl tricaprylate/caprate, from Abitec, Columbus OH), Crodamol™ GTC/C (medium chain triglyceride, from Croda, Cowick Hall, UK) or Labrafac™ CC (medium chain triglyides, from Gattefosse), Captex™ 500P (glyceryl triacetate i.e. triacetin, from Abitec), Capmul™ MCM (medium chain mono- and diglycerides, fromAbitec), Migyol™ 812 (caprylic/capric triglyceride, from Condea, Cranford NJ), Migyol™ 829 (caprylic/capric/succinic triglyceride, from Condea), Migyol™ 840 (propylene glycol dicaprylate/dicaprate, from Condea), Labrafil™ M1944CS (oleoyl macrogol-6 glycerides, from Gattefosse), Peceol™ (glyceryl monooleate, from Gattefosse) and Maisine™ 35-1(glyceryl monooleate, from Gattefosse). Of particular interest are the medium chain (about C_8 to C_{10}) triglyceride oils. These solvents frequently make up the predominant portion of the composition, i.e., greater than about 50%, usually greater than about 80%, for example about 95% or 99%. Adjuvants and additives may also be included with the solvents principally as taste-mask agents, palatability and flavoring agents, antioxidants, stabilizers, texture and viscosity modifiers and solubilizers.

Suspensions, in addition to the MTP inhibitor or the combination, may further comprise carriers such as suspending agents, e.g., ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, and tragacanth, or mixtures of these substances, and the like.

Conveniently, for administration to non-human animals, the MTP inhibitor (or combination) can be carried in the drinking water so that a therapeutic dosage of the compound is ingested with the daily water supply. The compound can be directly metered into drinking water, preferably in the form of a liquid, water-soluble concentrate (such as an aqueous solution of a water-soluble salt).

Conveniently, the MTP inhibitor (or combination) can also be added directly to the feed, as such, or in the form of an animal feed supplement.

The present invention has several advantageous veterinary features. For the pet owner or veterinarian who wishes to increase leanness and/or trim unwanted fat from pet animals, the instant invention provides the means by which this may be accomplished. For poultry, beef and swine breeders, utilization of the method of the present invention yields leaner animals that command higher sale prices from the meat industry.

Embodiments of the present invention are illustrated by the following Examples. It is to be understood, however, that the embodiments of the invention are not limited to the specific details of these Examples, as other variations thereof will be known, or apparent in light of the instant disclosure, to one of ordinary skill in the art.

Example 1

Formulation: composition per mL – Dirlotapide: 5mg
Medium chain triglyceride oil up to 1mL

Feed: Gilpa Trinkets complete dry food (Gilbertson & Page, Welwyn Garden City, UK), with a fat content of approximately 5, 10 or 15% (air dry matter as fed) was offered to all dogs at 1.2 maintenance energy requirements throughout the weight loss and retraining phase, and at 90% of what was eaten during the last period of recording feed consumption at the end of the retraining phase for the post treatment phase.

The efficacy and safety of dirlotapide at a starting dose of 0.1 mg/kg in the treatment of excessive body weight was determined in overweight Labrador dogs fed diets with varying fat contents for up to 52 weeks (for more details see J. GOSSELLIN, S. PEACHY, J.SHERINGTON, T.G. ROWAN, S. J. SUNDERLAND (2007); Evaluation of dirlotapide for sustained weight loss in overweight Labrador retrievers; Journal of Veterinary Pharmacology and Therapeutics 30 (s1), 55-65, incorporated herein by reference). The study consisted of three consecutive phases: the weight loss phase was up to 168 days and the dose adjusted to achieve a body weight loss of approximately 1-3% per week; the retraining phase was up to 196 days and the dose adjusted to maintain body weight ±5% body weight at the end of the weight loss phase and finally, the post treatment phase where no test article was administered and was up to 31 days.

Seventy two dogs were blocked by body condition score and body weight, and randomised to one of nine treatments: Placebo for 168 days (T1, T2 and T3, starting daily dose of 0.02 mL/kg for the first month of treatment), dirlotapide for up to 168 days followed by placebo for up to 196 days (T4, T5 and T6), or dirlotapide for up to 364 days (T7, T8 or T9). The dogs were acclimatised to the study housing, diet and environmental conditions for up to 39 days prior to day 0, the first day of treatment administration. The dogs were fed diets with varying fat contents based on air dry matter as fed: 5% fat diet (T1, T4 and T7), 10% fat diet (T2, T5 and T8), or 15% fat diet (T3, T6 and T9). The dogs were weighed, assessed for body condition, blood sampled, assessed for faecal consistency and clinically examined every 28 days throughout the

weight loss, retraining and post-treatment phases. In addition, the dogs were sampled for serum on day 24 or 25 of the weight loss phase, clinical observations performed on days 0 to 14, inclusive, and faecal consistency assessed on days 0 to 7 and days 13 and 14, of the weight loss phase. Dogs in T7, T8 and T9 were assessed for body composition on day –2, day 112 of the retraining phase and day 28 of the post treatment phase. On day 194 of the retraining phase, all remaining dogs (T4 to T9) were examined by an ophthalmologist for specific clinical signs of vitamin deficiencies. Subcutaneous adipose tissue samples were taken from all remaining dogs (T4 to T9) on the final day of the retraining phase, and final day of study (T7 to T9). All remaining dogs (T4 to T9) were examined by a veterinarian for specific clinical signs of vitamin deficiencies on the final day of the retraining phase. Blood was analysed for a full biochemistry profile, and samples from day 24/25 of the weight loss phase, and end of retraining and post treatment phases were analysed for vitamin A and E and prothrombin clotting times (prothrombin not assessed on day 24/25 samples).

Dirlotapide was safe and efficacious when administered for up to 52 weeks in the treatment of excessive body weight in dogs. All dirlotapide -treated dogs significantly (P=0.0001) lost weight during the weight loss phase (mean loss 0.86% per week) compared to placebo dogs (mean loss 0.02% per week). Loss of body weight was maintained in the dirlotapide -treated dogs during the retraining phase at a reduced rate (mean loss 0.2% per week), which was in contrast to the placebo dogs that gained 0.65% per week.

Compared to feed intakes during the acclimatisation period, dirlotapide-treated dogs had a significant decrease (P=0.0001) in feed intake during the weight loss phase compared to placebo-treated dogs (mean decrease of 17.1 vs. 0.9%).

During the combined weight loss and retraining phases the mean fat loss (based on body weight) in the dirlotapide -treated dogs was between 0.385 and 0.442% per week, whilst the mean lean mass change was between –0.0196 and +0.0021% per week confirming that lean mass was spared. None of the dirlotapide -treated dogs gained a body condition score during the weight loss or retraining phase, whereas dogs treated with placebo either gained or had no change in body condition score.

There were no serious suspected adverse drug experiences during the study period.

Dosing

The starting dose volume 0.02 mL/kg for the placebo dogs was equivalent to 0.1 mg/kg dirlotapide.

During the weight loss phase, the mean dose increased in all dirlotapide-treated groups (Figure 1) with the mean final dose ranging between 0.2 mg/kg and 0.6 mg/kg. The placebo-treated groups (T1 to T3) had a mean final dose volume ranging between 0.19 and 0.20 mL/kg, which was the maximum dose volume allowed. At the end of the weight loss phase, mean final dosages were significantly higher (P=0.0020; i.e. more dose increases were required) in dogs fed the 5% fat diet compared to dogs fed the 15% fat diet. Final mean dosage at the end of the weight loss phase was 0.50, 0.40 and 0.22 mg/kg initial body weight for five, ten and 15% fat diets, respectively.

Body Weight

There was a significant overall treatment effect (P=0.0001; Table 1) during the weight loss phase between dirlotapide and placebo-treated dogs (mean weekly body weight loss was 0.86% and 0.02%, respectively). There was a significant interaction (P=0.0066) between treatment and diet, and the treatment effect was significant with each diet (P=0.001 for all three fat diets).

The mean cumulative weight loss for dirlotapide-treated dogs during the weight loss phase was consistent over time and ranged between 18.4 and 22.3% (for the three fat diets; Figure 2), compared to the placebo dogs on the 10 and 15% fat diet whose weight remained constant (+1.5%). A cumulative decrease (4.2%) was observed in the placebo group on the 5% fat diet over the 168-day period.

There was a significant overall treatment effect (P=0.0001; Table 2) during the retraining phase between dirlotapide -treated dogs (mean weekly body weight loss 0.20%) and placebo-treated dogs which had previously been treated with dirlotapide during the weight loss phase (mean weekly body weight gain 0.65%). There was a significant interaction (P=0.0004) between treatment and diet, and the treatment effect was significant with each diet (P=0.0001 for all three fat diets).

Feed Intake

Feed intake during the weight loss, retraining and post treatment phases were compared to feed intakes during the acclimatisation period.

During the weight loss phase, dirlotapide -treated dogs had a significantly greater reduction (P=0.0001) in mean daily feed intake in contrast to the placebo dogs (Table 2; 17.1% and 0.9% respectively). There was no interaction between treatment and diet (P=0.3253).

Table 1 provides a summary of the weekly body weight changes (%) during the weight loss phase; Table 2 provides a summary of daily feed intake changes (%) during the weight loss phase as a percent of intake during the acclimatisation phase;
Figure 1 provides a summary of the dose volumes administered throughout the study period; and Figure 2 provides a summary of the mean cumulative body weight change (%) during the weight loss phase.

Table 1: Summary of weekly body weight changes (%) during the weight loss phase

	Placebo			Dirlotapide			Main Treatment Effect	
% Fat in Diet	5	10	15	5	10	15	Placebo	Dirlotapide
Body Weight Change [a]	-0.17	0.06	0.06	-0.80	-0.84	-0.94	-0.02	-0.86
CI [b]	-0.31; -0.04	-0.07; 0.20	-0.07; 0.20	-0.90; -0.71	-0.94; -0.74	-1.04; -0.85	-0.09; 0.06	-0.92; -0.81
Number of Animals	8	8	8	16	15	16	24	47

P=0.0001 for the effect of treatment

P=0.2423 for the effect of diet

P=0.0066 for the interaction between treatment and diet

[a] Least Square Mean percentage weekly body weight change

[b] 95% Confidence Interval (lower; upper)

Table 2: Summary of daily feed intake changes (%) during the weight loss phase as a percent of intake during the acclimatisation phase

	Placebo			Dirlotapide			Main Treatment Effect	
% Fat in Diet	5	10	15	5	10	15	Placebo	Dirlotapide
Change in Daily Feed Intake [a]	9.1	-6.1	-5.6	-11.7	-15.4	-24.3	-0.9	-17.1
CI [b]	-0.9; 19.0	-16.0; 3.9	-15.5; 4.4	-19.2; -4.2	-23.1; -7.7	-31.8; -16.8	-7.3; 5.6	-22.4; -11.9
Number of Animals	8	8	8	16	15	16	24	47

P=0.0001 for the effect of treatment

P=0.0044 for the effect of diet

P=0.3253 for the interaction between treatment and diet

[a] Least Square Mean percentage change of daily feed intake

[b] 95% Confidence Interval (lower; upper)

Example 2

Effects of Subchronic Administration of (4'-trifluoromethyl-biphenyl-2-carboxylic acid [2-(2H-[1,2,4]triazol-3-ylmethyl)-1,2,3,4-tetrahydro-isoquinolin-6-yl] amide) in Male Sprague-Dawley Rats

Animals: Male Sprague Dawley rats (215-225 g) (Charles River Laboratories, Wilmington, MA) were individually housed in hanging wire mesh cages at 21 ± 2°C with a 12:12 hour light:dark cycle. Rat were fed powdered purified diets providing either 10 or 45% of total calories as fat, with approximately equal caloric density (D01060501M, D01060502M Research Diets, New Brunswick, NJ) and had *ad libitum* access to water. All rats were acclimated to experimental diet and daily handling for 8 days prior to experimentation. All procedures were conducted in accordance with Institutional Animal Care and Use Committee guidelines and regulations.

Study Design: A total of 56 rats were used in this study. Rats were randomly assigned to treatment groups according to a randomization plan generated by www.randomization.com with twenty-eight rats assigned to the low fat diet, and twenty-eight assigned to the high fat diet. Glass food cups were fitted with a metal lid and a feeding screen to minimize spillage. Twenty-four hour baseline food intake was measured for 4 days prior to compound administration to ensure that baseline food intake was not significantly different between treatment groups. Food intake measurements continued through the third day of compound administration. Body weights were obtained on day –1, and daily from day 1 to 3 at approximately the same time each day. Rats received either 0.5% methylcellulose vehicle or a suspension of (4'-trifluoromethyl-biphenyl-2-carboxylic acid [2-(2H-[1,2,4]triazol-3-ylmethyl)-1,2,3,4-tetrahydro-isoquinolin-6-yl] amide) in 0.5% methylcellulose, at a dose of 0, 10, 30 or 100 mg/kg (10 mL/kg) for three consecutive days by standard oral gavage, at approximately 90 min before the onset of the dark cycle.

Treatment Group	Diet	Cumulative Weight Change (g)	*P* value versus vehicle
Vehicle	45%	24.60 ± 2.79	
10 mg/kg	45%	21.26 ± 2.53	> 0.050
30 mg/kg	45%	10.46 ± 3.10	< 0.050
100 mg/kg	45%	3.45 ± 2.46	< 0.050
Vehicle	10%	24.61 ± 2.59	
10 mg/kg	10%	25.95 ± 3.52	0.369
30 mg/kg	10%	23.95 ± 3.21	0.369
100 mg/kg	10%	18.43 ± 3.23	0.369

Table 3. Effect of three consecutive daily oral doses of vehicle or (4'-trifluoromethyl-biphenyl-2-carboxylic acid [2-(2H-[1,2,4]triazol-3-ylmethyl)-1,2,3,4-tetrahydro-isoquinolin-6-yl] amide) on cumulative body weight gain (mean ± SE) in male Sprague Dawley rats fed diets providing 45 or 10% of total calories from fat.

Example 3

Food intake and plasma endocrine mediators in response to dirlotapide administered at 10 mg/kg in normal rats fed either a high or low fat diet.

Animals

Male Sprague Dawley rats (initial weight 125-135 grams) (Charles River Laboratories, Wilmington, MA) were individually housed in hanging wire mesh cages at 21 ± 2°C with a 12:12 hr light:dark cycle. Rats were fed powdered purified diets providing either 10 or 45% of total calories from fat (low and high fat diet, respectively), with approximately equal caloric density (D01060501M, D01060502M, Research Diets, New Brunswick, NJ) and had *ad libitum* access to water. Glass food jars fitted with a metal lid and a feeding screen were secured to the front of individual cages with a metal spring or ring to facilitate food intake measurement. All rats were acclimated to diet and handling for a minimum of 5 days prior to experimentation. All studies were approved and conducted in

accordance with Institutional Animal Care and Use Committee guidelines and regulations.

Design

A total of 40 male rats were used in this experiment. Animals were randomly assigned to receive either the low or high fat diet, with 20 rats assigned to each group. Twenty-four hour food intake was measured for three consecutive days prior to compound administration (baseline) and during three consecutive days when rats received daily oral doses of compound or vehicle treatment. During the treatment period, 10 rats in each diet group were randomly assigned to receive either polyethylene glycol 400 (PEG 400): saline (80:20) vehicle or 10 mg/kg dirlotapide prepared in the same vehicle, both at dose volumes of 1 mL/kg body weight. Animals received treatments by standard oral gavage approximately 90 minutes prior to the onset of the dark cycle. Dirlotapide or vehicle was administered for 4 consecutive days with 24 hour food intake measured on the first three days and blood samples collected on the fourth day for analysis of compound exposure and potential plasma endocrine mediators of food intake.

Blood collection

Approximately 16 hrs after the fourth dose of vehicle or dirlotapide, rats were sacrificed by carbon dioxide inhalation and blood samples were collected via cardiac puncture. Blood was placed in tubes containing EDTA and Aprotinin (0.6TIU/mL, Phoenix Pharmaceuticals, Belmont, CA) to inhibit enzymatic protein degradation. Plasma samples were stored frozen at approximately – 20°C.

Analysis of Plasma Peptide YY, Cholescystokinin, Urocortin, Ghrelin and Pacreatic Polypeptide

Total plasma peptide YY concentrations (PYY 1-36 and PYY 3-36) were determined by radioimmunoassay (RIA) using a commercially available kit according to manufacturers' instructions (Phoenix Pharmaceuticals, Inc. Belmont, CA, # RK-059-03). Plasma cholescystokinin (CCK), urocortin, and ghrelin concentrations were determined by enzyme-linked immunoassay (ELISA) using commercially available kits according to

manufacturers' instructions (Phoenix Pharmaceuticals, Belmont, CA # EK-069-04, EK-019-15, EK-069-04). Pancreatic polypeptide (PP) concentrations were determined by Linco Research Inc., St Charles MD by RIA methods.

RESULTS

Administration of dirlotapide at 10 mg/kg for 3 consecutive days resulted in robust inhibition of mean daily food intake by 43% and decreased body weight gain by 56% compared to vehicle treated rats, when animals were fed a high fat diet (Figure 4). A smaller, but also significant decrease in food intake (13%) was observed in rats fed the low fat diet and treated with dirlotapide compared to vehicle controls.

Plasma concentrations of several known satiety factors in response to vehicle or dirlotapide administration for 4 consecutive days are shown in Table 4. Humoral endocrine mediators were measured in plasma from rats on the high fat diet only. Plasma PYY concentrations of rats receiving dirlotapide were significantly elevated by 370% compared to vehicle controls.

Table 4. Plasma concentrations (± SEM) of satiety factors of high fat diet fed rats receiving 3 consecutive daily doses of vehicle or dirlotapide at 10 mg/kg.

* Significantly different from vehicle control.

Mediator	**High Fat and Vehicle**	**High Fat and Dirlotapide**
Peptide YY (pM)	11.8 ± 1.6	55.4 ± 4.4*
Leptin (ng/mL)	8.3± 1.4	1.8 ± 0.5*
Pancreatic Polypeptide (pg/mL)	165.1 ± 13.0	130.0 ± 13.4
CCK (ng/mL)	1.2 ± 0.1	1.0 ± 0.1
Urocortin (ng/mL)	3.2 ± 0.23	3.9 ± 0.45
Ghrelin (ng/mL)	2.5 ± 0.26	2.6 ± 0.22

Example 4

The weight loss efficacy and safety of dirlotapide administered once daily to obese dogs presented as veterinary patients was evaluated in a randomized study that was conducted in two phases: In the first phase the study was placebo-controlled and twenty-eight obese dogs (body condition score (BCS) ≥ 7 on a 9-point scale) were enrolled. Ten (6 male and 4 female) placebo dogs (T1) received an initial dosage of 0.036 mL/lb medium chain triglyceride (MCT) oil vehicle and eighteen (6 male and 12 female) dirlotapide-treated dogs (T2) received an initial dosage of 0.4 mg/kg (0.18 mg/lb) dirlotapide once daily. The dose was adjusted by 10 or 20% at day 14 and 28 to produce between 1% to 3% weight loss per week during 56 days of treatment. In the second phase, eight T2-treated dogs continued on treatment until day 84 (T2-extension) and eight placebo-treated dogs received dirlotapide at an initial dosage of 0.2 mg/kg (0.09 mg/lb) that was adjusted monthly for 84 days (T3). There were no control animals for the second phase of the study. Body weights and BCS were recorded on approximately days 0, 14, and then monthly thereafter. The correlation between the % change in body weight from day 0 to each subsequent day and the % dietary fat the dog was eating was calculated for each treatment.

Dogs were fed a complete and balanced commercial diet during the study but the dietary fat was not prescribed by the protocol. Dietary fat, as a percentage of metabolizable energy, varied from 16% to 45% for T1, 22% to 51% (T2) and 16% to 45% (T3). For T3, there was a good correlation at day 28 (r=0.8), day 56 (r=0.8) and day 84 (r=0.9) between dietary fat and weight loss efficacy (higher fat as a % of kilocalories in the diet resulted in greater weight loss). This effect was not observed for T2 at day 28 (r=0.1) when many dogs were losing > 3% body weight weekly but was suggested at day 56 (r=0.4).

Claims

1. A method of treating a subject suffering from obesity or related eating disorders and/or reducing food consumption, the method comprising administering to the subject a therapeutically effective amount of an MTP inhibitor in conjunction with an increased-fat diet.

2. The the use of an MTP inhibitor in the manufacture of a medicament for treating a subject suffering from obesity or related eating disorders and/or reducing food consumption, wherein a therapeutically effective amount of an MTP inhibitor is administered in conjunction with an increased-fat diet.

3. A method or use according to claim 1 or claim 2 wherein said increased-fat diet has a fat content of at least approximately 10% (based on air dry matter as fed).

4. A method or use according to claim 3 wherein said increased-fat diet has a fat content of at least approximately 15% (based on air dry matter as fed).

5. A method or use according to claim 1 or claim 2 wherein said increased-fat diet provides approximately 24% or greater than 24% of total calories from fat.

6. A method or use according to claim 5 wherein said increased-fat diet provides approximately 34% or greater than 34% of total calories from fat.

7. A method or use according to any preceding claim wherein the MTP inhibitor is an intestinal-acting MTP inhibitor.

8. A method or use according to any preceding claim wherein the MTP inhibitor is selected from the group consisting of:

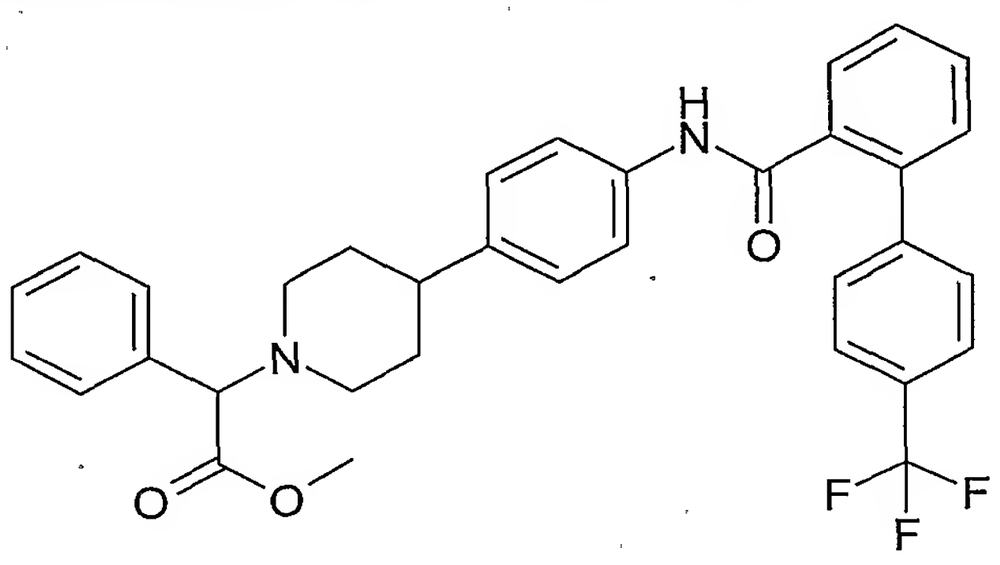
dirlotapide;

mitratapide;

(S)-2-[(4'-trifluoromethyl-biphenyl-2-carbonyl)-amino]-quinoline-6-carboxylic acid (pentylcarbamoyl-phenyl-methyl)-amide;

(S)-2-[(4'-tert-butyl-biphenyl-2-carbonyl)-amino]-quinoline-6-carboxylic acid {[(4-fluoro-benzyl)-methyl-carbamoyl]-phenyl-methyl}-amide;

(S)-2-[(4'-tert-butyl-biphenyl-2-carbonyl)-amino]-quinoline-6-carboxylic acid [(4-fluoro-benzylcarbamoyl)-phenyl-methyl]-amide;
(S)-2-[(4'-isopropoxy-biphenyl-2-carbonyl)-amino]-quinoline-6-carboxylic acid {[(4-fluoro-benzyl)-methyl-carbamoyl]-phenyl-methyl}-amide;



R256918 ; or
(4'-trifluoromethyl-biphenyl-2-carboxylic acid [2-(2H-[1,2,4]triazol-3-ylmethyl)-1,2,3,4-tetrahydro-isoquinolin-6-yl] amide);
or a pharmaceutically acceptable salt, hydrate or solvate thereof.

9. A method or use according to claim 8 wherein the MTP inhibitor is dirlotapide.

10. A method or use according to any preceding claim optionally followed by administration of at least one step-wise, escalating dosage of the MTP inhibitor and, optionally, followed by a weight maintenance/management or retraining phase.

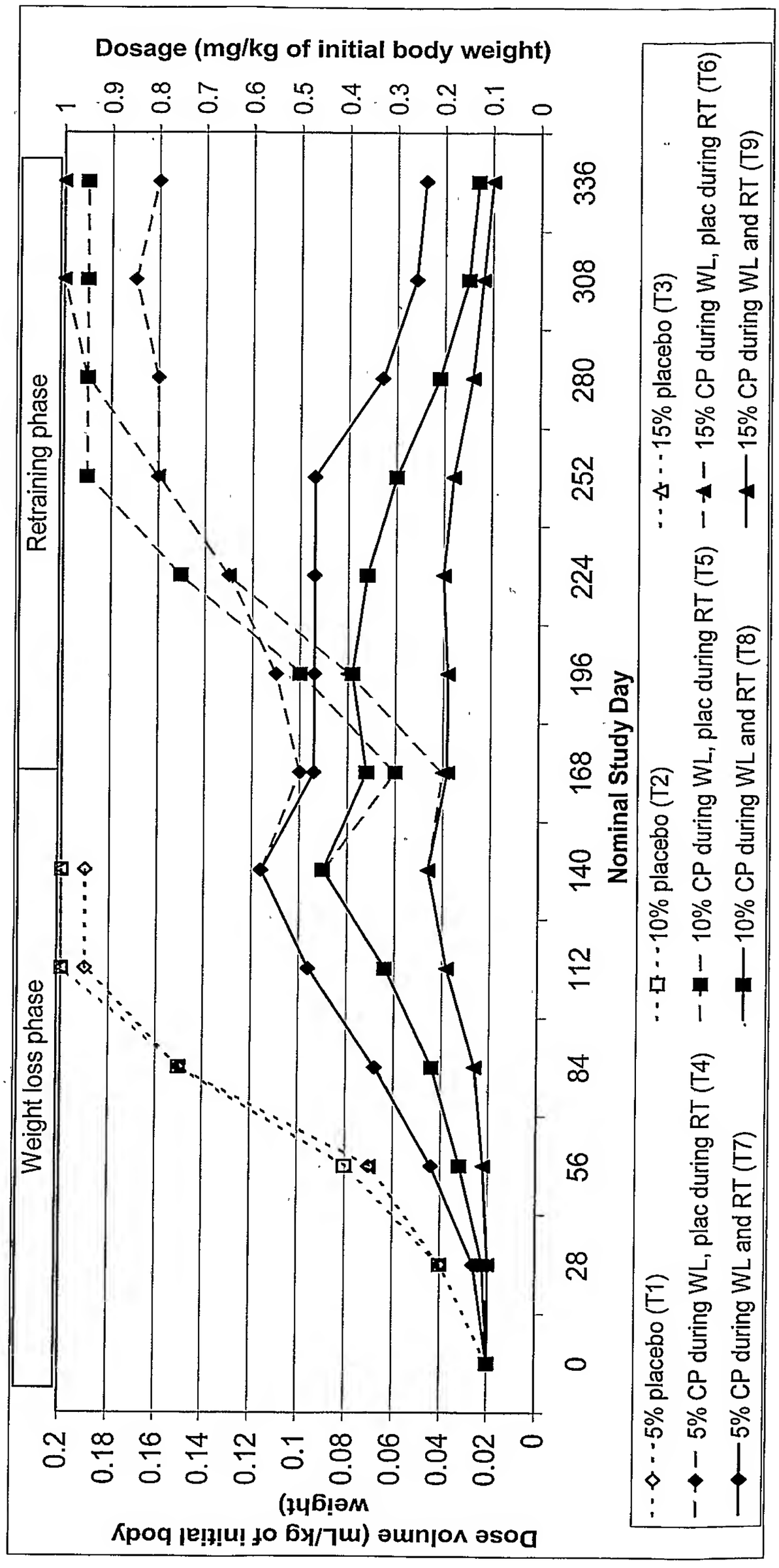


Figure 1: Dose volumes throughout the study period

WL = Weight loss phase

RT = Retraining phase

CP = Dirlotapide

plac = Placebo

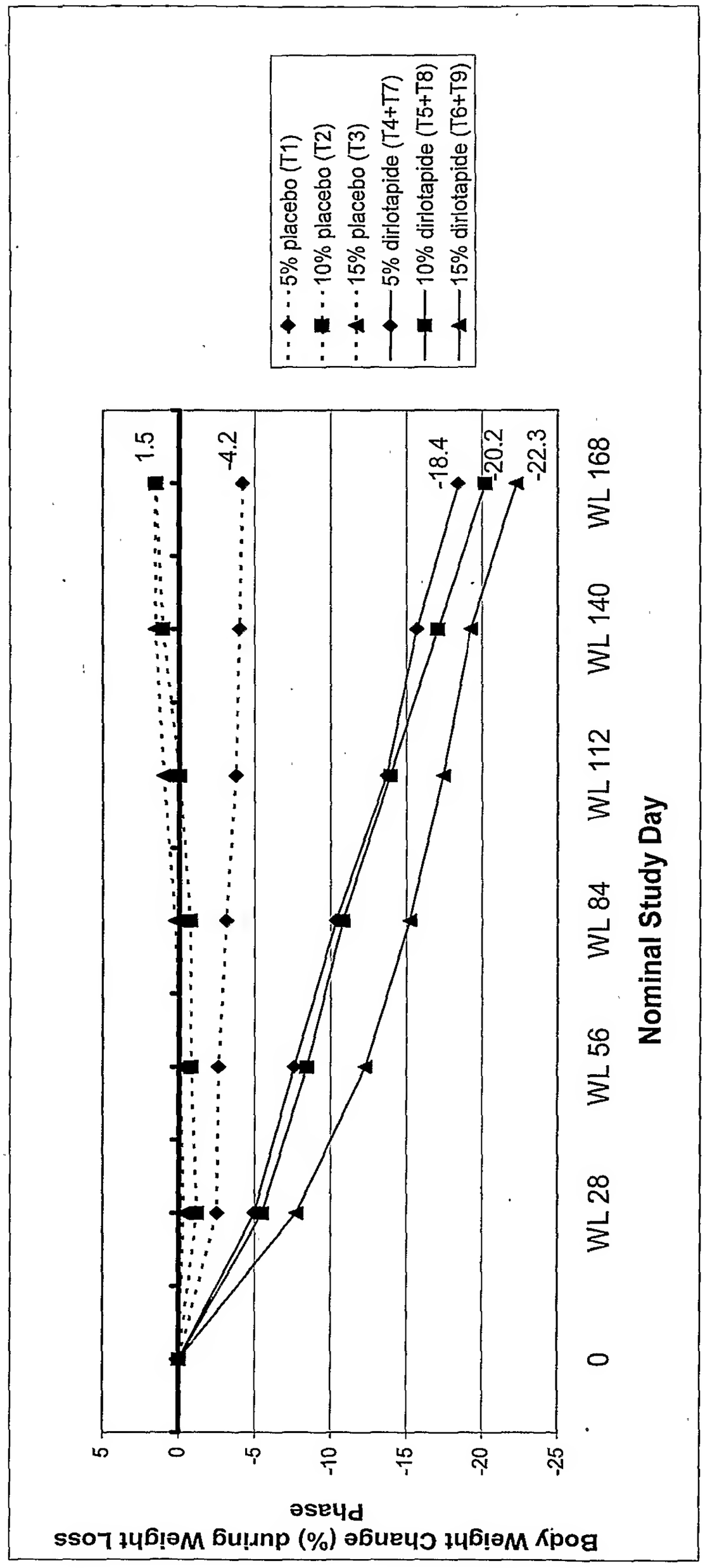


Figure 2: Mean cumulative body weight change (%) during the weight loss phase

WL = Weight loss phase
28, 56 etc. = Day of weight loss phase

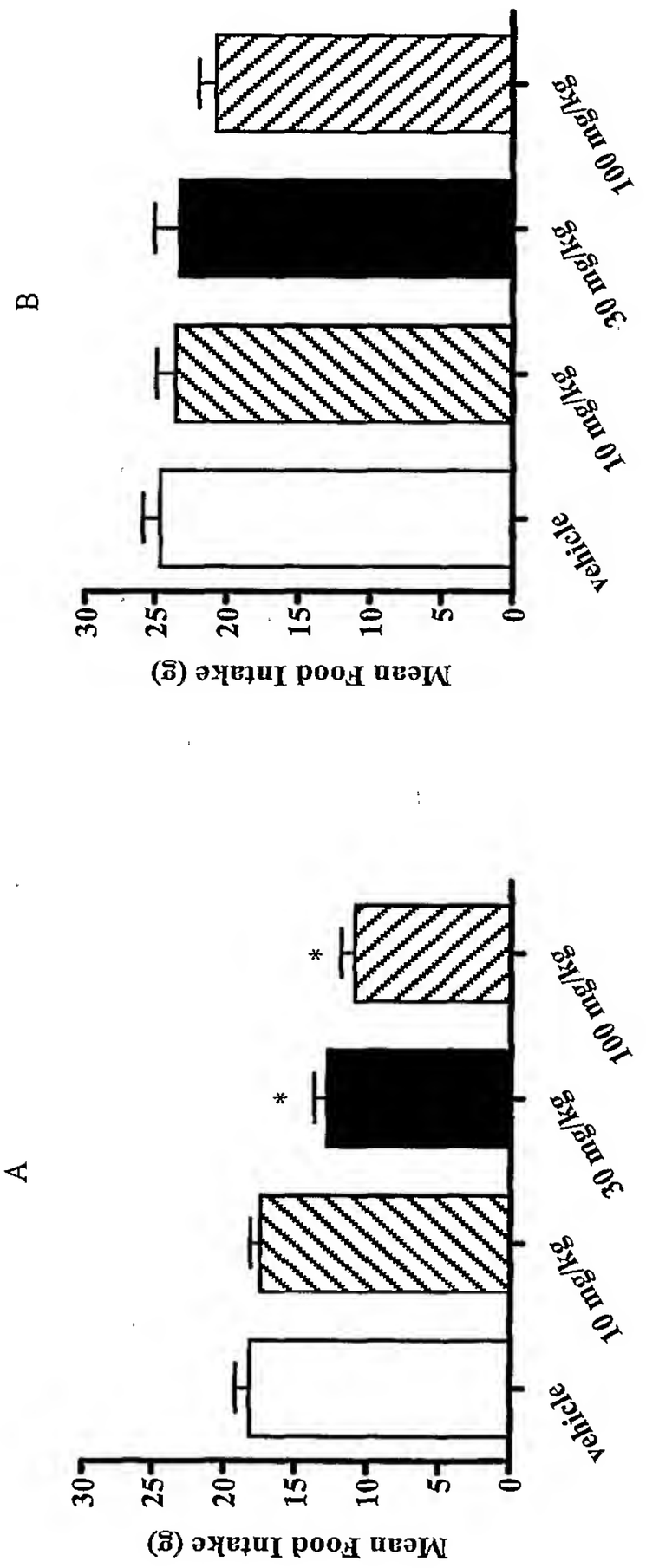
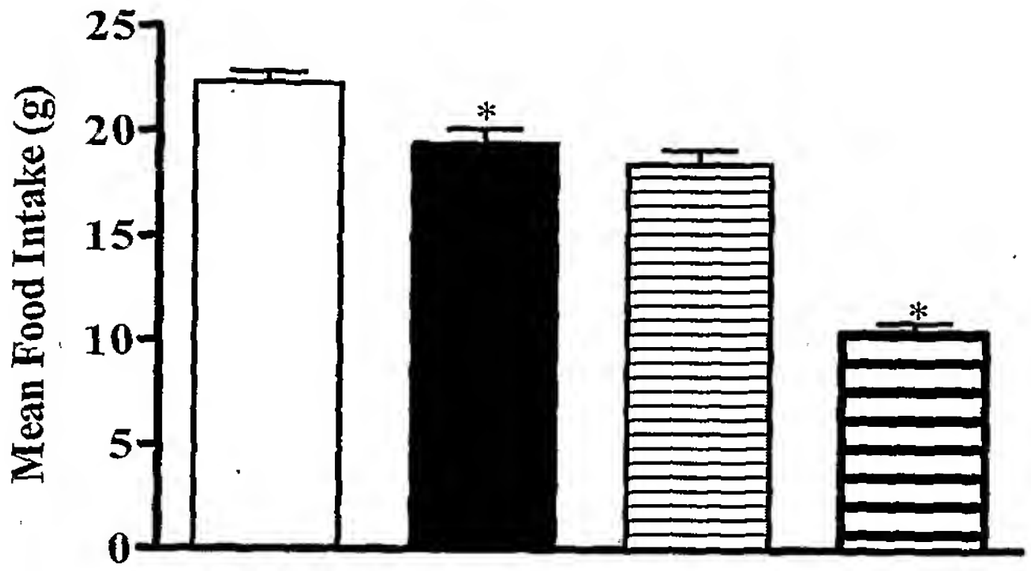


Figure 3. Effects of (4'-trifluoromethyl-biphenyl-2-carboxylic acid [2-(2H-[1,2,4]triazol-3-ylmethyl)-1,2,3,4-tetrahydro-isoquinolin-6-yl] amide) on daily food intake (mean ± SE) in male Sprague-Dawley rats. Rats were fed powdered purified diets providing either 45% (A) or 10% (B) of total calories from fat. Rats received vehicle or (4'-trifluoromethyl-biphenyl-2-carboxylic acid [2-(2H-[1,2,4]triazol-3-ylmethyl)-1,2,3,4-tetrahydro-isoquinolin-6-yl] amide) at a dose of 10, 30 or 100 mg/kg (n = 7) for 3 consecutive days.
* $P < 0.05$ compared to vehicle.

Figure 4



Mean (± SEM) daily food intake of rats on a high or low fat diet during the 3 day treatment period. Empty bar = low fat vehicle placebo, solid black bar = low fat dirlotapide, small horizontal slashes = high fat vehicle, thick horizontal slashes = high fat dirlotapide. * Significantly different from appropriate diet vehicle.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2007/003855

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/4045 A61K31/437 A61K31/451 A61K31/4709 A61K31/496
A61P3/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/270655 A1 (SWICK ANDREW G [US] ET AL) 30 November 2006 (2006-11-30) paragraphs [0011], [0079], [0080], [0084], [0090]; claims 1,4	1-10

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
8 May 2008	23/05/2008

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2007/003855

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2006270655 A1	30-11-2006	NONE	